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GENETIC RELATEDNESS OF LENTIL (*Lens culinaris* L.) GERMPLASM BY USING SSR MARKERS

U. K. S. Kushwaha[1], S. K. Ghimire[1], N. K. Yadav [2] and B. R. Ojha[1]

[1]Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal

[2]Nepal Agricultural Research Council, Khumaltar, Nepal

Corresponding author email: kushujjwal@gmail.com

Abstract

Ninety six lentil accessions from different origins were collected from National Grain Legume Research Program, Rampur; Regional Agriculture Research Station, Nepalgunj and National Agriculture Genetic Resource Center, Khumaltar, Lalitpur. Among them; four lines were Nepal Local, forty two lines were Nepal Cross; forty seven lines were ICARDA Line and finally three lines were Indian Line. All ninety six accessions were analysed by DNA fingerprinting using thirty three selected polymorphic SSR markers. The characterization was performed in Biotechnology Unit, Nepal Agricultural Research Council, Khumaltar, Lalitpur by using standard protocols. Molecular variance analysis showed that 14 % genetic variation was found between population and 86 % genetic variation was found within population with estimated variance 0.23 between population and 1.35 within population. Highest genetic distance (9) was found between landrace ILL-7979 and RL-20. In the same way, highest Nei genetic distance (0.03) between population was shown by population 1 and population 4; and lowest genetic distance were observed within the same population accessions. The heterozygosity was probably due to the introgression of genes or duplication of microsatellite motif during the breeding and or the course of lentil line evolution. All the accessions included in this study displayed significant amount of genetic variability and genetic relatedness due to different center of origin and different genetic constitutions. The diversity detected in this study may constitute the new materials for future systematic lentil breeding programs.

Keywords: lentil, germplasm, characterization, genotypes, gene

Introduction

The knowledge of genetic diversity and association of characters with yield is of great importance to the breeder for making an improvement of quantitative characters. Molecular marker is used for estimating genetic variation at population level and among closely related species (Nienhuis *et al.,* 1995). Several classes of molecular markers have been developed showing that lentil has relatively low levels of genetic variation (Eujay l*et al.,* 1997; Sonnante and Pignone, 2001). Plant descriptors coupled with molecular markers provide a valid evidence of diversity as these are least affected by environmental fluctuations (Ahmad *et al.,* 1997; Jha and Ohri, 1996; Margale *et al.,* 1995).

Lentil (*Lens culinaris* Medik.subsp. *culinaris*) is an important principal cool season pulse crop of the Indian Subcontinent, the Middle East, North America, North Africa and West Asia (Erskine, 1996). Nepal has ha area 1,87,437altogether of lentil 1,51,758with d per hectare kg yiel 810metric ton productivity and M)oAD .(2011 ,The crop has developed into a range of varieties adapted to diverse growing areas and cultural preferences, and containing unique nutritional compositions, colors, shapes and tastes. A lot of lentil land races, primitive races, indigenous races and wild races are still available in Nepal but they have not been studied properly .The genetic relatedness of lentil based on molecular level has not been studied yet in Nepal. Thus the yield attributing traits, disease resistance traits, insect pest resistance traits, abiotic stress tolerance traits and quality traits have not been identified and, cause delay in breeding for developing elite lines. Now a day the importance of lentil in Nepal is increasing due to its high nutritive value, important components of Nepalese diet, increased internal consumption and exportable commodity to foreign countries. Thus, there is an urgent need to increase the overall production and productivity of this crop through varietal improvement and suitable agronomic practices under rice-maize cropping systems in Nepal. Before initiation of lentil breeding activities there is urgent need to characterize, evaluate lentil germplasm available to us. Therefore present study was conducted

with an objective of selecting divergent parents based on genetic distance for future lentil breeding programme.

Materials and Methods

Diverse lentil germplasm were collected from National Grain Legume Research Program (NGLRP), Rampur; Regional Agricultural Research Station (RARS), Nepalgunj and National Agriculture Genetic Resource Center (NAGRC), Khumaltar. Collected accessions comprised fourlocal line/Nepalese native (Nepal Local): pop1; forty two NGLRP, Rampur crossed (Nepal Cross):pop2; forty seven ICARDA (ICARDA Line): pop3; and three from India (IndianLine): pop4 . The list of the collected germplasm is given in Table 1. Thirty three polymorphic microsatellites marker were used for PCR based on the results of previous report (Hamwieh *et al.,* 2005, 2009). The list of polymorphic markers, their name, sequence information, annealing temperature and amplification size are given in Table 2.

Table 1: 96 lentil materials used in this study based on source of origin

DNA SN	Variety name	Source of origin	DNA SN	Variety name	Source of origin
1	LN-0135	Nepal Local	13	ILL-10071	ICARDA
25	LN-0136	Nepal Local	14	ILL-9924	ICARDA
91	Arial	Nepal Local	15	ILL-6465	ICARDA
95	Khajura Masuro- 2	Nepal Local	16	ILL-9926	ICARDA
2	RL-45	Nepal Cross	17	ILL-6458	ICARDA
3	RL-67	Nepal Cross	18	ILL-1020	ICARDA
4	RL—49	Nepal Cross	19	ILL-6811	ICARDA
5	RL-79	Nepal Cross	20	HUL-57	ICARDA
7	RL-56	Nepal Cross	21	Sagun	ICARDA
8	RL-68	Nepal Cross	22	M.Bharati	ICARDA
9	RL-8	Nepal Cross	23	ILL-7162	ICARDA
10	X94S-48	Nepal Cross	24	ILL-7723	ICARDA
34	RL-4	Nepal Cross	26	ILL-3768	ICARDA
44	RL-60	Nepal Cross	28	ILL-8006	ICARDA (BM-4)
47	RL-70	Nepal Cross	29	ILL-7537	ICARDA
48	RL-73	Nepal Cross	31	IL-1	ICARDA
53	RL-71	Nepal Cross	32	ILL-7979	ICARDA
54	NR 2001-72-3	Nepal Cross	33	ILL-7715	ICARDA
57	RL-75	Nepal Cross	35	ILL-6467	ICARDA
58	RL-35	Nepal Cross	36	ILL-7164	ICARDA
59	RL-43	Nepal Cross	37	ILL-3490	ICARDA
60	RL-69	Nepal Cross	38	ILL-6419	ICARDA
61	RL-44	Nepal Cross	40	ILL-3111	ICARDA
62	RL-42	Nepal Cross	41	ILL-2527	ICARDA
63	RL-76	Nepal Cross	42	FLIP 2006-99L	ICARDA
64	RL-26	Nepal Cross	43	FLIP 95-1L	ICARDA
65	RL-41	Nepal Cross	45	FLIP 2009-60L	ICARDA
66	RL-39	Nepal Cross	46	FLIP 04-60L (ILL-10013)	ICARDA
67	RL-58	Nepal Cross	6	ILL-3338	ICARDA
68	RL-62	Nepal Cross	50	ILL-6021	ICARDA
69	RL-47	Nepal Cross	51	FLIP 05-24L (ILL-10045)	ICARDA
70	RL-80	Nepal Cross	52	FLIP 05-24L (ILL-10065)	ICARDA
71	RL-21	Nepal Cross	55	FLIP 2008-7L	ICARDA
72	RL-23	Nepal Cross	56	FLIP 2009-54L	ICARDA
75	RL-94	Nepal Cross	73	FLIP 05-52L (ILL-10073)	ICARDA
78	NR 2001-71-4	Nepal Cross	74	ILL-6260	ICARDA
79	RL-74	Nepal Cross	76	X39S-66L	ICARDA
80	RL-20	Nepal Cross	77	ILL-10134	ICARDA
81	RL-25	Nepal Cross	83	ILL-10068	ICARDA
82	RL-95	Nepal Cross	87	ILL-7664	ICARDA
84	RL-22	Nepal Cross	88	Digger	ICARDA
85	RL-38	Nepal Cross	89	Bari Musuro-4	ICARDA
86	RL-5	Nepal Cross	92	ILL-6458	ICARDA
90	NX 9901 – 1	Nepal Cross	93	X 95583	ICARDA
96	RL 28	Nepal Cross	94	FLIP 2009 – 59L (ILL 10716)	ICARDA
97	RL-78	Nepal Cross	27	DPL-62	India
11	ILL-2712	ICARDA	30	WBL-77	India
12	ILL-1970	ICARDA	39	LG-12	India

Table 2. List of forward and reverse SSR primers used for lentil characterization, with annealing temperature and expected size.

S.N.	SSR No.	Forward	Reverse	Annealing temp. (Tm) used for PCR (°C)	expected size (bp)
1	SSR 34-2	CGGCGGATGAAACTAAAG	CATTTCCTTCACAAACCAAC	53	185
2	SSR 66	GGTAGTGGTGAGCAATGAC	GCATCACTGCAACAGACC	55	253
3	SSR 90	CCGTGTACACCCCTAC	CGTCTTAAAGAGAGTGACAC	55	181
4	SSR 132RN	CCAGAACAAACGTAAACC	CTATCGCATATGAGTGAAC	52	330
5	SSR 191	GCAAATTTCTTGGTCTACAC	GGGCACAGATTCATAAGG	53	238
6	SSR 197	CACCAATCACCAACACAC	GAGCTGTGAAGTCTTATCTG	54	173
7	SSR 207	GAGAGATACGTCAGAGTAG	GATTGTGCTTCGGTGGTTC	55	227
8	SSR 230	CCAACAACAATTCACCATAC	AACATTGTACTGAGACGTG	53	251
9	SSR 33	CAAGCATGACGCCTATGAAG	CTTTCACTCACTCAACTCTC	56	289
10	SSR 19	GACTCATACTTTGTTCTTAGCAG	GAACGGAGCGGTCACATTAG	58	250
11	SSR 48	CATGGTGGAATAGTGATGGC	CTCCATACACCACTCATTCAC	57	165
12	SSR 96	GTTATCTTCCAGCGTC	GATATACAATCAGAGATG	49	210
13	SSR 99	GGGAATTTGTGGAGGGAAG	CCTCAGAATGTCCCTGTC	57	161
14	SSR 107	GCGGCGAGCAAATAAAT	GGAGAATAAGAGTGAAATG	51	161
15	SSR 113	CCGTAAGAATTAGGTGTC	GGAAAATACGGTGGAAAG	51	211
16	SSR 119	GAACTCAGTTTCTCATTG	GAACATATCCAATTATCATC	49	266
17	SSR 124	GTATGTGACTGTATGCTTC	GCATTGCATTTCACAAACC	52	174
18	SSR 130	CCACGTATCTGACTGTATG	GAAAGAGAGGCTCAAACTTG	55	196
19	SSR 156	GTACATTGAACAGCATCATC	CAAATGGGCATGAAAGGAC	53	176
20	SSR 167	CACATATGAAGATTGGTCAC	CATTTATGTCTCACACACAC	54	160
21	SSR 199	GTGTGCATGGTGTGTG	CCATCCCCCTCTATC	51	182
22	SSR 204	CACGACTATCCCACTTG	CTTACTTTCTTAGTGCTATTAC	53	186
23	SSR 212-1	GACTCATTGTTGTACCC	GCGAGAAGAATGGTTG	50	181
24	SSR 213	CACTCGCACCTCTTATG	GAAATTGTCTCTTAGCAAG	51	151
25	SSR 309-2	GTATGTCGTTAACTGTCGTG	GAGGAAGGAAGTATTCGTC	50	182
26	SSR 317-1	GTGGGTGTAATTATTGCTAC	GTATCAAACTTATGGTGAAATC	53	308
27	SSR 317-2	CACGTAACATCTTGCTTATG	GTAGCAATAATTACACCCAC	53	120
28	SSR 323	AGTGACAACAAAATGTGAGT	GTACCTAGTTTCATCATTG	51	250
29	SSR 336	GTGTAACCCAACTGTTCC	GGCCGAGGTTGTAACAC	54	253
30	SSR 183	GCTCGCATTGGTGAAAC	CATATATAGCAGACCGTG	52	119
31	SSR 202	CAACCTCACTTACCTTAC	GCTCTTTATCATCATTCTAC	52	220
32	SSR 28	GAGGGCATAAATTCAGATTC	GGACAACGCACATTTGATG	53	383
33	SSR 72	CAAACAGTACAAGGAAAGGAG	CTGACTGAGCTGCTTGAAC	55	253

DNA fingerprintingwas conducted with SSR markers. This fingerprinting was performed in Biotechnology unit, Nepal Agricultural Research Council, Khumaltar, Lalitpur. Lentil DNA extraction was done by Modified CTAB method (Doyle and Doyle, 1987) using standard protocol followed by DNA quantification, PCR amplification, gel separation and scoring of gel separated bands using standard protocol. The amplified products were scored as bands on visualization on gel on UV illuminator. Only the reliable bands were included in analysis. The presence of bands was scored as "1" and absence of band was scored as "0". The respective data analysis, data entry and processing was carried out by using Microsoft Excel 2007. Percentage of molecular variance and genetic distance were found out by GenAlEx^{x}6.5b3.xls.

Results and Discussion

Molecular variance analysis

Molecular variance analysis for genetic diversity of ninety six genotypes of lentil was carried out by GenAlEx6.5b3.xls. 14 % genetic variation was found between population and 86 % genetic variation was found within populations (figure 1). Estimated variance between population was 0.228 with 14.42 % and within population was 1.358 with 85.57 % out of 1.581 with 100% with PhiPT 0.144 (Table 3). This showed that high genetic relatedness were between population and far relatedness were within population.

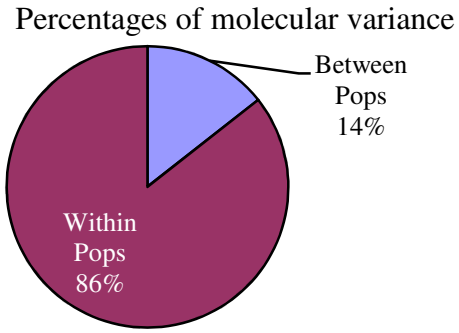

Fig. 1: Percentage of molecular variance between and within population for 96 lentil accessions.

Table 3. Summary of AMOVA table with estimated variance among and within population for 96 lentil accessions

Source	Df	SS	MS	Est. Var.	%
Between Pops	3	16.430	5.477	0.228	14%
Within Pops	92	124.476	1.353	1.353	86%
Total	95	140.906		1.581	100%
Stat	Value	P (rand >= data)			
PhiPT	0.144	0.010			

Where PhiPT = AP / (WP + AP) = AP / TOT Key: AP = Est. Var. Between Pops, WP = Est. Var. Within Pops (PhiPT max=0.918; Phi'PT=0.157 P(rand >= data) 0.010)

Table 4. Pairwise population matrix of nei genetic distance for 96 lentil accessions with four population

	Pop1	Pop2	Pop3	Pop4
Pop1	0.000			
Pop2	0.015	0.000		
Pop3	0.032	0.019	0.000	
Pop4	0.033	0.029	0.015	0.000

Table 5. Pairwise population matrix of nei unbiased genetic distance for 96 lentil accessions with four population

	Pop1	Pop2	Pop3	Pop4
Pop1	0.000			
Pop2	0.000	0.000		
Pop3	0.016	0.017	0.000	
Pop4	0.006	0.016	0.001	0.000

Genetic distance

The pairwise population matrix showed that highest (0.033) Nei Genetic Distance was found between pop 1 and pop 4 and lowest was found within the same population i.e. pop1, pop2, pop3 and pop4. Similarly, pop3 and pop2 had highest (0.017) Nei Unbiased Genetic Distance and lowest distance was found within the same population (Table 4 and 5). Pop1 (Nepal Local) and pop2 (Indian Line) had highest genetic distance which might be due to different center of origin and different genetic constitutions. Similarly, genetic relatedness were found within the populations which might be due to same center of origin and similiar genetic constitutions.

Highest genetic distance (9) was found between landrace 32 (ILL-7979) and 80 (RL-20) calculated from GenAlEx6.5b3.xls. The highest and lowest level of genetic distance was 0.027273 and 0 respectively. The difference between the highest and the lowest inter genotypic distance indicates the moderate variability among the 96 genotypes of lentil.

Conclusion

Highest genetic distance (9) was found between landrace ILL-7979 and RL-20. Similarly, high genetic relatedness were found within the same population which might be due to same center of origin and similar genetic constitutions. In the same way high genetic distance were found between Nepal Line and Indian Line which might be due to different center of origin and different genetic constitutions. The level of genetic relatedness detection largely depends on the type of molecular markers, nature of SSR repeat motif, number of SSR markers and the genetic relatedness of the lentil germplasm to be analysed. All ninety six genotypes involved in the study exhibited wide range of genetic variability due to different center of origin, different genetic constitution. The genetic relatedness detected in this study may constitute the foundation for future systematic lentil breeding programs.

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